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Docket No.: SOL.003.P
Express Mail No.: EU720332434US

OFFICE ACTION RESPONSE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: David A. Schwartz
Serial No.: 09/815,978
Filed: 22 March 2001
Group Art Unit: 1654
Examiner: J. E. Russel

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For: "HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS"

APPLICANT'S RESPONSE PURSUANT TO 37 C.F.R. §1.111

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

The following is Applicant's response to the Office action dated 9 January 2003 (Paper No.: 14).

INTRODUCTORY COMMENTS

In response to the Examiner Restriction Requirement Applicant preliminarily elected Group I with traverse requesting that Groups I and II be joined as having overlapping subject matter. The argument was not found persuasive by the Examiner and Group I comprising claims 1, 2, 22-30 and 40-44 was elected for prosecution and claims 3-21, 31-39 and 45-53 have been withdrawn without prejudice.

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AMENDMENTS TO THE CLAIMS

Please add the following new claims:

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54. (New) A compound of formula I

$$B\text{-}R\text{-}A\text{-}NHNH_2 \cdot HX$$

I

wherein;

A is -NH(C=O)-, -NH(C=S)-, -NHNH(C=O)- or -NHNH(C=S)-;

B is an amino reactive moiety;

R is an aliphatic divalent group having any combination of the following groups, which are combined in any order: cycloalkylene, $C(R^{10})_2$, $-C(R^{10})=C(R^{10})-$, $>C=C(R^{12})(R^{13})$, $>C(R^{12})(R^{13})$, $-C\equiv C-$, O, $S(G)_a$, $P(J)_b(R^{10})$, $P(J)_b(L)(R^{10})$, $N(R^{10})$, $>N^+(R^{12})(R^{13})$ and C(L); where a is 0, 1 or 2, b is 0, 1, 2 or 3; G is O or $N(R^{10})$; J is S or O; and L is S, O or $N(R^{10})$; each R^{10} is a monovalent group independently selected from hydrogen and $M^1(R^{14})$; each M^1 is a divalent group independently having any combination of the following groups, which groups are combined in any order: a direct link, arylene, heteroarylene, cycloalkylene, $C(R^{15})_2$, $-C(R^{15})=C(R^{15})-$, $>C=C(R^{12})(R^{13})$, $>C(R^{12})(R^{13})$, $-C\equiv C-$, O, $S(G^1)_a$, $P(J)_b(R^{15})$, $P(J)_b(L)(R^{15})$, $N(R^{15})$, $N(C=O)R^{15}$, $>N^+(R^{12})(R^{13})$ and C(L); where a is 0, 1 or 2; b is 0, 1, 2 or 3; G^1 is O or $N(R^{15})$, J is S or O; and L is S, O or $N(R^{15})$; R^{14} and R^{15} are each independently selcted from the group consisting of hydrogen, halo, pseudohalo, cyano, azido, nitro, $Si(R^{16})(R^{17})(R^{18})$, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroarakenyl, heteroalkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl; hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy and $N(R^{19})(R^{20})$; R^{19} and R^{20} are each independently selected from the

group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl; or (ii) R^{12} and R^{13} together form alkylene, alkenylene or cycloalkylene; R^{16}, R^{17} and R^{18} are each independently a monovalent group selected from hydrogen, alkyl, alkenyl, alkynyl, haloalkyl, haloalkyoxy, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroalkenyl, heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy, and $N(R^{19})(R^{20})$; and

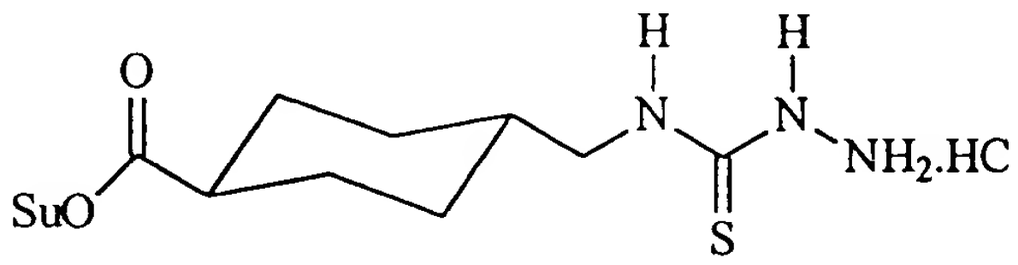
R^{10}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{20} can be substituted with one or more substituents each independently selected from Z, wherein Z is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, hydroxy, $S(O)_hR^{30}$, $N(R^{30})(R^{31})$, $C{=}OOR^{30}$, $C{=}O(R^{30})$, $C{=}ON(R^{30})(R^{31})$, $O(C{=}O)N(R^{30})(R^{31})$, $N(R^{30})(C{=}O)R^{31}$, alkoxy, aryloxy, heteroaryl, heterocyclyl, heteroaryloxy, heterocyclyloxy, aralkyl, aralkenyl, aralkynyl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, aralkoxy, heteroaralkoxy, alkoxycarbonyl, carbamoyl, thiocarbamoyl, alkoxycarbonyl, carboxyaryl, halo, pseudohalo, haloalkyl, and caboximido; h is 0, 1 or 2; and R^{30} and R^{31} are each independently selected from the group consisting of hydrogen, halo, psuedohalo, cyano, azido, nitro, trialkylsilyl, dialkylarylsilyl, alkyldiarysilyl, triarylsilyl, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl, arakenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocylclylalkenyl, heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteraralkoxy, amino, amido, alkylamino, dialkylamino, alkylarylamino, diarylamino and arylamino; and

X is a negative counterion.

55. (New) The compound according to claim 54, wherein R is, or is a combination of, a saturated straight chain of 1 to 20 carbon atoms, a chain of 2

to 2000 ethyleneoxide moieties or a saturated or unsaturated carbocyclic moiety of 3 to 20 carbon atoms.

56. (New) The compound of the formula:



57. (New) The compound according to claim 54, wherein X is a halide or trifluoroacetate.

58. (New) The compound according to claim 54, wherein B is an amino reactive moiety is a succinimidyl ester, a hydroxybenzotriazolyl ester or a pentafluorophenol ester.

59. (New) A conjugate comprising the compound according to claim 54 bound to a natural or synthetic biological molecule.

60. (New) The conjugate according to claim 59, wherein the natural or synthetic molecule is selected from the group consisting of a protein, a glycoprotein, a peptide, an oligonucleotide, an RNA a DNA and a synthetic polymer.

61. (New) The conjugate according to claim 60, wherein the protein is an antibody.

62. (New) A method of immobilizing a natural or synthetic biological molecule, comprising:

(a) preparing the conjugate according to claim 59; and

(b) applying the conjugate to a surface wherein the surface has at least one carbonyl moiety for a time and under conditions such that the hydrazine moiety of the conjugate reacts with the at least one carbonyl moiety of the surface forming a hydrazone bond to the surface.

63. (New) A method of crosslinking a natural or synthetic biological molecule, comprising:

(a) preparing the conjugate according to claim 59; and

(b) applying the conjugate to a surface wherein the surface has at least one amino reactive moiety for a time and under conditions such that the conjugate reacts with the at least one amino reactive moiety of the surface forming a bond to the surface.

64. (New) A method of crosslinking a natural or synthetic biological molecule, comprising:

(a) preparing the conjugate according to claim 59; and

(b) mixing the conjugate with a natural or synthetic molecule wherein the molecule has at least one carbonyl moiety for a time and under conditions such that the hydrazine moiety of the conjugate reacts with the at least one carbonyl moiety of the molecule forming a hydrazone bond to the molecule.

65. (New) The method according to claim 62, wherein the surface is selected from the group consisting of glass, polymer, latex and colloidal metal.

66. (New) The method according to claim 64, wherein the natural or synthetic biological molecule is selected from the group consisting of a protein, a glycoprotein, a peptide, an oligonucleotide, an RNA and a DNA.

67. (New) The method according to claim 66, wherein the protein is an antibody.

68. (New) A surface prepared by the method according to claim 62.

69. (New) A composition prepared by the method according to claim 64.

70. The compound according to claim 1 wherein A is -NH(C=O)- B is an amino reactive moiety and R is $-O(C=O)(C_6H_{10})CH_2-$.

Please cancel claims 1-53 without prejudice.

AMENDMENTS TO THE SPECIFICATION

Please amend the specification by replacing the paragraph on page 4 line 23 through page 5 line 1 with:

"Thus, the reagents provided herein are aliphatic and aromatic crosslinking compounds that possess (I) a thiol or amine reactive group; and (ii) a hydrazino, oxyamino or carbonyl group. Thiol reactive groups are moieties that react directly with sulfhydryls groups forming stable thioether bonds. These thiol reactive groups include, but are not [limted] limited to, maleimido, α-bromoacetamido and pyridyldisulfides. Amino reactive moieties are those that react directly with amine moieties forming amide bonds. These amino reactive groups include, but are not limited to, N-hydroxysuccinimidyl, p-nitrophenyl, pentafluorophenyl and N-hydroxybenzotriazolyl esters."

Please replace the previously submitted sequence listing with the enclosed new sequence listing on compact disc containing the statement:

"The contents of this sequence listing information recorded in computer readable form is identical to the written (On paper or compact disc) sequence listing and contains no new matter."

Please amend the specification by replacing the paragraph on page 51 line 25 through page 52 line 5 with:

"A 25-mer phosphodiester oligonucleotide modified to incorporate C6-aminolinker (Glen Research amino-C6 amidite) was prepared (5'-NH_2-$(CH_2)_6$-ttt ttt tag cct aac tga tgc cat g-3'; SEQ ID NO.: 1 MW 7791 g/mol, 229.5 OD/μmol; Trilink Bio Technologies, Inc. San Diego, CA). The oligonucleotide was dissolved in conjugation buffer (100mM phosphate, 150 mM sodium chloride, pH 7.4) to a concentration of 0.92 OD/μL. To a solution of oligonucleotide (64 μL; 2 mg) was added DMF (32 μL). A solution of SANH (EXAMPLE 2; 3.8 mg) in DMF (100 μL) was prepared. An aliquot of the SANH/DMF solution (18.8 μL; 10 equivalents)

was added to the oligonucleotide solution and the reaction allowed to incubate at room temperature overnight. The reaction was monitored by C18 RP-HPLC (solution A: 50 mM triethylammonium acetate, Solution B: acetonitrile-gradient 0-50% A over 30 min.; 50-80% over 10 min.: 80-0% over 5 min.) The hydrazine-modified oligonucleotide was deprotected and purified using a Millipore 5K MWCO ultrafree diafiltration device by diluting the reaction mixture with 100 mM acetate, pH 4.7 and concentrating in the diafiltration device. The retentate was further washed with buffer (2 X 400µL). The oligonucleotide was quantified by A_{260} assay and the hydrazine incorporation was determined using the p-nitrobenzaldehyde assay described in EXAMPLE 9."

AMENDMENTS TO DRAWING FIGURES

Please replace Figure 5 with the enclosed replacement Figure 5.